American Journal of Respiratory and Critical Care Medicine/AJRCCM

Impact of T2R38 receptor polymorphisms on Pseudomonas aeruginosa infection in cystic fibrosis

Journal:	*American Journal of Respiratory and Critical Care Medicine*
Manuscript ID	Blue-201711-2365LE.R1
Manuscript Type:	LE - Letter-to-the-Editor
Date Submitted by the Author:	n/a
Complete List of Authors:	Turnbull, Andrew; Imperial College London Murphy, Ronan; Imperial College London, National Heart and Lung Institute Behrends, Volker; University of Roehampton, Department of Life Sciences; University of Roehampton Lund-Palau, Helena; Imperial College London, National Heart & Lung Institute Simbo, Ameze; Imperial College London, National Heart and Lung Institute Mariveles, Myril; Imperial College London, National Heart and Lung Institute Alton, Eric; Imperial College, Bush, Andrew; Imperial College and Royal Brompton Hospital, London Shoemark, Amelia; Royal Brompton Hospital, EM Unit; Imperial College London, Gene Therapy Davies, Jane; Imperial College London, National Heart and Lung Institute
Subject Category:	9.17 Cystic Fibrosis: Translational & Clinical Studies < LUNG DISEASES, 10.06 Host Defenses to Microbial Pathogens < MICROBIOLOGY AND PULMONARY INFECTIONS, 7.18 Mucosal Immunity of the Respiratory Tract < IMMUNOLOGY AND INFLAMMATION
Keywords:	Cilia, Taste receptor, type 2, Quorum sensing, Mucociliary clearance

Impact of T2R38 receptor polymorphisms on *Pseudomonas aeruginosa* infection in cystic fibrosis

Andrew R. Turnbull[1,2], Ronan Murphy[1], Volker Behrends[3], Helena Lund-Palau[1], Ameze Simbo[1], Myril Mariveles[1], Eric W.F.W. Alton[1], Andrew Bush[1,2], Amelia Shoemark[1,2,4], Jane C. Davies[1,2]

[1]National Heart & Lung Institute, Imperial College London, United Kingdom.

[2]Paediatric Respiratory Medicine, Royal Brompton & Harefield NHS Foundation Trust, London, UK.

[3]Health Science Research Centre, Department of Life Sciences, University of Roehampton, London, UK.

[4]Department of Clinical and Molecular Medicine, University of Dundee, Dundee, UK.

Corresponding author:

Andrew R. Turnbull,

National Heart & Lung Institute,

Imperial College,

London SW3 6LR,

a.turnbull14@imperial.ac.uk

Author contributions:

Conception and design: ART, AS[1,2,4] and JCD. Data collection: ART, AS[1,2,4], VB, RM, HLP, AS[1] and MM. Analysis and interpretation: ART, AS[1,2,4], VB and JCD. Manuscript drafting: ART, AS[1,2,4] and JCD. Editing and approval: all authors.

Running title: T2R38 receptor polymorphisms in cystic fibrosis

Description number: Cystic Fibrosis: Translational & Clinical Studies

Manuscript word count: 1036

To the editor:

The T2R38 bitter taste receptor on respiratory epithelia detects *P. aeruginosa N*-acyl-L-homoserine lactones (AHLs). *In vitro,* T2R38 activation by AHLs initiates calcium-mediated increases in nitric oxide production and ciliary beat frequency, dependent on polymorphisms in the *TAS2R38* gene (1). In patients with chronic rhinosinusitis (CRS), *TAS2R38* genotype is proposed to modify mucosal responses to *P. aeruginosa* (1).

Polymorphisms in the *TAS2R38* gene result in two high-frequency haplotypes, associated with taste perception of the bitter compound phenylthiocarbamide (2). The 'taster' haplotype codes proline-alanine-valine (PAV); the 'non-taster' haplotype codes alanine-valine-isoleucine (AVI), at positions 49, 262, and 296 in the receptor protein. Responses to AHLs *in vitro* are greatest in PAV/PAV epithelial cells, and this genotype is reported to be protective against *P. aeruginosa* in the sinonasal airway (1).

P. aeruginosa is the most frequently isolated respiratory pathogen in cystic fibrosis (CF), and chronic infection is associated with accelerated rates of disease progression. Determining the impact of *TAS2R38* polymorphisms on *P. aeruginosa* infection in CF could have implications for patient risk stratification and, as naturally-occurring and synthetic agonists to T2R38 are already in clinical use (3), could identify promising therapeutic targets.

We characterized T2R38 localization in the CF airway and investigated the hypothesis that *TAS2R38* polymorphisms would modify prevalence and impact of *P. aeruginosa* infection in CF. Some of the results of these studies have previously been reported as abstracts (4, 5).

Methods

Nasal and/or bronchial brushings were obtained from 4 CF children undergoing bronchoscopy and 4 healthy adult controls. T2R38 localization was evaluated by immunocytochemistry with antibodies to T2R38 and ciliary proteins, as described previously (6). Slides were imaged with a Zeiss LSM-510 confocal microscope and colocalization was quantified using the JACoP plug-in for ImageJ (7).

DNA was extracted from blood from 271 subjects with CF aged >6yrs and subjected to PCR for the common *TAS2R38* polymorphisms (rs713598, rs1726866, and rs10246939). *P. aeruginosa* infection status was categorised in patients with ≥3 respiratory cultures during 2014, according to Leeds criteria (8), as chronic (>50% positive), intermittent (≤50% positive), free (previous *P. aeruginosa* but none for >12 months), or never. Clinical data was obtained from each patient's 2014 annual assessment.

Cryo-preserved *P. aeruginosa* isolates from *TAS2R38*-genotyped patients (matched for age and FEV_1) were revived in Luria-Bertani broth in triplicate and filter-sterilized. Quantitative analysis of *N*-butanoyl-L-homoserine lactone (C4-HSL) and *N*-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL) was performed by liquid chromatography with tandem mass spectrometry. Limits of detection and limits

of quantification were defined as signal:noise ratios of 3:1 and 10:1 respectively, as previously described (9).

Power calculations predicted 250 patients would provide 80% power to detect a difference in chronic *P. aeruginosa* infection of ≥20% in PAV/PAV compared to other genotypes at α of 5%. Analysis of *P. aeruginosa* infection by *TAS2R38* genotype was by Chi-squared analysis and logistic regression. Graphpad Prism 7 and SPSS 23 were used and the null hypothesis was rejected at $p<0.05$.

Ethical review committees (02-019 and 10/H0504/9) approved the protocol, and written consent was obtained from subjects or their parent/guardian.

Results

T2R38 immunostaining was present in all nasal (n=3) and bronchial (n=3) samples from CF patients and all nasal samples (n=4) from healthy controls. T2R38 stained proximally to acetylated α-tubulin (ciliary microtubules) and γ-tubulin (ciliary basal bodies), and colocalized with rootletin (ciliary rootlets) in CF and control cells (figure). Thresholded Manders' correlation coefficients (mean ± SD of 4 cells) for T2R38 and rootletin were 0.91 ± 0.07, 0.90 ± 0.08 and 0.90 ± 0.04 for control nasal, CF nasal, and CF bronchial cells respectively, indicating that ≥90% of green (rootletin) pixels were positive for red (T2R38).

Of 271 CF patients, 225 had the common AVI/AVI (74), AVI/PAV (110) or PAV/PAV (41) genotypes and ≥3 respiratory cultures during 2014. Between *TAS2R38* genotype groups there was no significant difference in median age, sex, or

proportion of p.Phe508del *CFTR* mutations. There was no association between *TAS2R38* genotype and *P. aeruginosa* infection status (P=0.46) (table). In the logistic regression model with 'intermittent and chronic', and 'never and free' groups as dependent variables, and age, sex, *CFTR* genotype and *TAS2R38* genotype as independent variables, only age was associated with intermittent or chronic *P. aeruginosa* infection (odds ratio 1.05, 95% CI 1.03-1.07). There was no association between *TAS2R38* genotype and *P. aeruginosa* infection status when the PAV/PAV genotype was compared against AVI/AVI or AVI/PAV genotypes.

Among patients with intermittent or chronic *P. aeruginosa* infection (n=141) there was no difference by *TAS2R38* genotype in median percent-predicted FEV_1 (AVI/AVI 54.0%, AVI/PAV 62.0%, PAV/PAV 53.5%, p=0.3) or in the proportion of patients who isolated mucoid *P. aeruginosa* (AVI/AVI 69%, AVI/PAV 60%, PAV/PAV 68%, p=0.5). In 18 *P. aeruginosa* isolates from *TAS2R38*-genotyped patients there was no difference by genotype in the proportion of isolates in which C4-HSL or 3-oxo-C12-HSL were below the limit of quantification (p=0.8).

Discussion

We have identified T2R38 in CF nasal and bronchial epithelium, where it localizes to the ciliary rootlet in the same distribution as in non-CF epithelia. Previous studies report T2R38 localization ranging from the ciliary tip (10) to below the ciliary base (1, 11). Our experiments demonstrate that in fresh, non-cultured cells, T2R38 colocalizes with rootletin, a structural component of the ciliary rootlet, originating from the ciliary basal body and extending toward the nucleus (12).

In this study of 225 children and adults with CF we have found no association between *TAS2R38* genotype and *P. aeruginosa* infection status, within the range of difference that our study was powered to detect. Our results show only age to be associated with intermittent or chronic infection, consistent with CF registry data (13). Among patients with intermittent or chronic infection, the lack of any difference in spirometry or prevalence of mucoid *P. aeruginosa* adds further evidence to the lack of a protective effect of the PAV/PAV genotype. Finally, in a small sample of clinical isolates we observed no relationship between *TAS2R38* genotype and AHL profiles, suggesting that polymorphisms in this receptor are not exerting a selective pressure on *P. aeruginosa* in the CF lung.

Our results indicate that *TAS2R38*-related differences in sinonasal immunity do not translate to clinically relevant changes in the CF airway, where mucociliary clearance is significantly impaired. We suggest there to be no prognostic value of *TAS2R38* genotyping in patients with CF, nor do our findings indicate the T2R38 receptor to be a promising drug target in CF mucosal immunity.

Acknowledgements

This project was supported by the NIHR Respiratory Disease Biomedical Research Unit at the Royal Brompton and Harefield NHS Foundation Trust and Imperial College London. The *P. aeruginosa* clinical isolate repository was established as part of the Strategic Research Centre for Pseudomonal infection in CF, funded by the Cystic Fibrosis Trust (UK). The Facility for Imaging by Light Microscopy (FILM) at Imperial College London is part-supported by funding from the Wellcome Trust (grant 104931/Z/14/Z) and BBSRC (grant BB/L015129/1).

References

1. Lee RJ, Xiong G, Kofonow JM, Chen B, Lysenko A, Jiang P, Abraham V, Doghramji L, Adappa ND, Palmer JN, Kennedy DW, Beauchamp GK, Doulias PT, Ischiropoulos H, Kreindler JL, Reed DR, Cohen NA. T2R38 taste receptor polymorphisms underlie susceptibility to upper respiratory infection. *The Journal of clinical investigation* 2012; 122: 4145-4159.
2. Kim UK, Jorgenson E, Coon H, Leppert M, Risch N, Drayna D. Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide. *Science (New York, NY)* 2003; 299: 1221-1225.
3. Meyerhof W, Batram C, Kuhn C, Brockhoff A, Chudoba E, Bufe B, Appendino G, Behrens M. The molecular receptive ranges of human TAS2R bitter taste receptors. *Chemical senses* 2010; 35: 157-170.
4. Turnbull A, Lund-Palau H, Murphy R, Simbo A, Wong K, Bush A, Alton E, J D. The T2R38 bitter taste receptor as a modifier of host response to Pseudomonas aeruginosa in cystic fibrosis: does T2R38 genotype impact on clinical infection? *Pediatric pulmonology* 2016; 51: S81-S114.
5. Turnbull A, Shoemark A, Palau HL, Murphy RA, Simbo A, Bush A, Alton EW, Davies JC. Determining the impact of the T2R38 bitter taste receptor on P. aeruginosa infection in the cystic fibrosis airway. *Journal of Cystic Fibrosis* 2017; 16: S88.
6. Shoemark A, Frost E, Dixon M, Ollosson S, Kilpin K, Patel M, Scully J, Rogers AV, Mitchison HM, Bush A, Hogg C. Accuracy of Immunofluorescence in the Diagnosis of Primary Ciliary Dyskinesia. *American journal of respiratory and critical care medicine* 2017.
7. Bolte S, Cordelieres FP. A guided tour into subcellular colocalization analysis in light microscopy. *J Microsc* 2006; 224: 213-232.
8. Lee TWR, Brownlee KG, Conway SP, Denton M, Littlewood JM. Evaluation of a new definition for chronic Pseudomonas aeruginosa infection in cystic fibrosis patients. *Journal of Cystic Fibrosis* 2003; 2: 29-34.
9. Ortori CA, Dubern JF, Chhabra SR, Camara M, Hardie K, Williams P, Barrett DA. Simultaneous quantitative profiling of N-acyl-L-homoserine lactone and 2-alkyl-4(1H)-quinolone families of quorum-sensing signaling molecules using LC-MS/MS. *Analytical and bioanalytical chemistry* 2011; 399: 839-850.
10. Shah AS, Ben-Shahar Y, Moninger TO, Kline JN, Welsh MJ. Motile cilia of human airway epithelia are chemosensory. *Science (New York, NY)* 2009; 325: 1131-1134.
11. Yan CH, Hahn S, McMahon D, Bonislawski D, Kennedy DW, Adappa ND, Palmer JN, Jiang P, Lee RJ, Cohen NA. Nitric oxide production is stimulated by bitter taste receptors ubiquitously expressed in the sinonasal cavity. *American journal of rhinology & allergy* 2017; 31: 85-92.
12. Yang J, Liu X, Yue G, Adamian M, Bulgakov O, Li T. Rootletin, a novel coiled-coil protein, is a structural component of the ciliary rootlet. *The Journal of cell biology* 2002; 159: 431-440.
13. Kerem E, Viviani L, Zolin A, MacNeill S, Hatziagorou E, Ellemunter H, Drevinek P, Gulmans V, Krivec U, Olesen H. Factors associated with FEV1 decline in cystic

fibrosis: analysis of the ECFS patient registry. *The European respiratory journal* 2014; 43: 125-133.

FIGURE LEGEND

Figure. Confocal microscopy images of nasal epithelial cells from a subject with CF. Cells were stained with antibodies to T2R38 (red), and acetylated α-tubulin (A) or rootletin (B) (both stained green). Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) (blue). Colocalized antibodies appear yellow in the merged images. Epithelial cell morphology is shown by differential interference contrast (DIC) images. T2R38 stains proximally to acetylated α-tubulin (ciliary microtubules) and colocalizes with rootletin (ciliary rootlets).

Antibodies used in these immunocytochemistry assays were: T2R38 (AB130503, Abcam, UK), acetylated α-tubulin (T6793, Sigma, UK), and rootletin (SC-374056, Santa Cruz Biotechnology, USA).

Table. *P. aeruginosa* infection category by *TAS2R38* genotype

	AVI/AVI (n=74)	AVI/PAV (n=110)	PAV/PAV n=41
Never, n(%)	4 (5)	4 (4)	3 (7)
Free, n(%)	21 (28)	36 (32)	16 (39)
Intermittent, n(%)	11 (15)	26 (24)	8 (20)
Chronic, n(%)	38 (51)	44 (40)	14 (34)

Definition of abbreviations: AVI = alanine-valine-isoleucine; PAV = proline-alanine-valine.

Impact of T2R38 receptor polymorphisms on *Pseudomonas aeruginosa* infection in cystic fibrosis

Andrew R. Turnbull[1,2], Ronan Murphy[1], Volker Behrends[3], Helena Lund-Palau[1], Ameze Simbo[1], Myril Mariveles[1], Eric W.F.W. Alton[1], Andrew Bush[1,2], Amelia Shoemark[1,2,4], Jane C. Davies[1,2]

[1]National Heart & Lung Institute, Imperial College London, United Kingdom.

[2]Paediatric Respiratory Medicine, Royal Brompton & Harefield NHS Foundation Trust, London, UK.

[3]Health Science Research Centre, Department of Life Sciences, University of Roehampton, London, UK.

[4]Department of Clinical and Molecular Medicine, University of Dundee, Dundee, UK.

Corresponding author:

Andrew R. Turnbull,

National Heart & Lung Institute,

Imperial College,

London SW3 6LR,

a.turnbull14@imperial.ac.uk

Author contributions:

Conception and design: ART, AS[1,2,4] and JCD. Data collection: ART, AS[1,2,4], VB, RM, HLP, AS[1] and MM. Analysis and interpretation: ART, AS[1,2,4], VB and JCD. Manuscript drafting: ART, AS[1,2,4] and JCD. Editing and approval: all authors.

Running title: T2R38 receptor polymorphisms in cystic fibrosis

Description number: Cystic Fibrosis: Translational & Clinical Studies

Manuscript word count: 1036

To the editor:

The T2R38 bitter taste receptor on respiratory epithelia detects *P. aeruginosa N*-acyl-L-homoserine lactones (AHLs). *In vitro,* T2R38 activation by AHLs initiates calcium-mediated increases in nitric oxide production and ciliary beat frequency, dependent on polymorphisms in the *TAS2R38* gene (1). In patients with chronic rhinosinusitis (CRS), *TAS2R38* genotype is proposed to modify mucosal responses to *P. aeruginosa* (1).

Polymorphisms in the *TAS2R38* gene result in two high-frequency haplotypes, associated with taste perception of the bitter compound phenylthiocarbamide (2). The 'taster' haplotype codes proline-alanine-valine (PAV); the 'non-taster' haplotype codes alanine-valine-isoleucine (AVI), at positions 49, 262, and 296 in the receptor protein. Responses to AHLs *in vitro* are greatest in PAV/PAV epithelial cells, and this genotype is reported to be protective against *P. aeruginosa* in the sinonasal airway (1).

P. aeruginosa is the most frequently isolated respiratory pathogen in cystic fibrosis (CF), and chronic infection is associated with accelerated rates of disease progression. Determining the impact of *TAS2R38* polymorphisms on *P. aeruginosa* infection in CF could have implications for patient risk stratification and, as naturally-occurring and synthetic agonists to T2R38 are already in clinical use (3), could identify promising therapeutic targets.

We characterized T2R38 localization in the CF airway and investigated the hypothesis that *TAS2R38* polymorphisms would modify prevalence and impact of *P. aeruginosa* infection in CF. Some of the results of these studies have previously been reported as abstracts (4, 5).

Methods

Nasal and/or bronchial brushings were obtained from 4 CF children undergoing bronchoscopy and 4 healthy adult controls. T2R38 localization was evaluated by immunocytochemistry with antibodies to T2R38 and ciliary proteins, as described previously (6). Slides were imaged with a Zeiss LSM-510 confocal microscope and colocalization was quantified using the JACoP plug-in for ImageJ (7).

DNA was extracted from blood from 271 subjects with CF aged >6yrs and subjected to PCR for the common *TAS2R38* polymorphisms (rs713598, rs1726866, and rs10246939). *P. aeruginosa* infection status was categorised in patients with ≥3 respiratory cultures during 2014, according to Leeds criteria (8), as chronic (>50% positive), intermittent (≤50% positive), free (previous *P. aeruginosa* but none for >12 months), or never. Clinical data was obtained from each patient's 2014 annual assessment.

Cryo-preserved *P. aeruginosa* isolates from *TAS2R38*-genotyped patients (matched for age and FEV_1) were revived in Luria-Bertani broth in triplicate and filter-sterilized. Quantitative analysis of *N*-butanoyl-L-homoserine lactone (C4-HSL) and *N*-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL) was performed by liquid chromatography with tandem mass spectrometry. Limits of detection and limits

of quantification were defined as signal:noise ratios of 3:1 and 10:1 respectively, as previously described (9).

Power calculations predicted 250 patients would provide 80% power to detect a difference in chronic *P. aeruginosa* infection of ≥20% in PAV/PAV compared to other genotypes at α of 5%. Analysis of *P. aeruginosa* infection by *TAS2R38* genotype was by Chi-squared analysis and logistic regression. Graphpad Prism 7 and SPSS 23 were used and the null hypothesis was rejected at $p<0.05$.

Ethical review committees (02-019 and 10/H0504/9) approved the protocol, and written consent was obtained from subjects or their parent/guardian.

Results

T2R38 immunostaining was present in all nasal (n=3) and bronchial (n=3) samples from CF patients and all nasal samples (n=4) from healthy controls. T2R38 stained proximally to acetylated α-tubulin (ciliary microtubules) and γ-tubulin (ciliary basal bodies), and colocalized with rootletin (ciliary rootlets) in CF and control cells (figure). Thresholded Manders' correlation coefficients (mean ± SD of 4 cells) for T2R38 and rootletin were 0.91 ± 0.07, 0.90 ± 0.08 and 0.90 ± 0.04 for control nasal, CF nasal, and CF bronchial cells respectively, indicating that ≥90% of green (rootletin) pixels were positive for red (T2R38).

Of 271 CF patients, 225 had the common AVI/AVI (74), AVI/PAV (110) or PAV/PAV (41) genotypes and ≥3 respiratory cultures during 2014. Between *TAS2R38* genotype groups there was no significant difference in median age, sex, or

proportion of p.Phe508del *CFTR* mutations. There was no association between *TAS2R38* genotype and *P. aeruginosa* infection status (P=0.46) (table). In the logistic regression model with 'intermittent and chronic', and 'never and free' groups as dependent variables, and age, sex, *CFTR* genotype and *TAS2R38* genotype as independent variables, only age was associated with intermittent or chronic *P. aeruginosa* infection (odds ratio 1.05, 95% CI 1.03-1.07). There was no association between *TAS2R38* genotype and *P. aeruginosa* infection status when the PAV/PAV genotype was compared against AVI/AVI or AVI/PAV genotypes.

Among patients with intermittent or chronic *P. aeruginosa* infection (n=141) there was no difference by *TAS2R38* genotype in median percent-predicted FEV_1 (AVI/AVI 54.0%, AVI/PAV 62.0%, PAV/PAV 53.5%, p=0.3) or in the proportion of patients who isolated mucoid *P. aeruginosa* (AVI/AVI 69%, AVI/PAV 60%, PAV/PAV 68%, p=0.5). In 18 *P. aeruginosa* isolates from *TAS2R38*-genotyped patients there was no difference by genotype in the proportion of isolates in which C4-HSL or 3-oxo-C12-HSL were below the limit of quantification (p=0.8).

Discussion

We have identified T2R38 in CF nasal and bronchial epithelium, where it localizes to the ciliary rootlet in the same distribution as in non-CF epithelia. Previous studies report T2R38 localization ranging from the ciliary tip (10) to below the ciliary base (1, 11). Our experiments demonstrate that in fresh, non-cultured cells, T2R38 colocalizes with rootletin, a structural component of the ciliary rootlet, originating from the ciliary basal body and extending toward the nucleus (12).

In this study of 225 children and adults with CF we have found no association between *TAS2R38* genotype and *P. aeruginosa* infection status, within the range of difference that our study was powered to detect. Our results show only age to be associated with intermittent or chronic infection, consistent with CF registry data (13). Among patients with intermittent or chronic infection, the lack of any difference in spirometry or prevalence of mucoid *P. aeruginosa* adds further evidence to the lack of a protective effect of the PAV/PAV genotype. Finally, in a small sample of clinical isolates we observed no relationship between *TAS2R38* genotype and AHL profiles, suggesting that polymorphisms in this receptor are not exerting a selective pressure on *P. aeruginosa* in the CF lung.

Our results indicate that *TAS2R38*-related differences in sinonasal immunity do not translate to clinically relevant changes in the CF airway, where mucociliary clearance is significantly impaired. We suggest there to be no prognostic value of *TAS2R38* genotyping in patients with CF, nor do our findings indicate the T2R38 receptor to be a promising drug target in CF mucosal immunity.

Acknowledgements

This project was supported by the NIHR Respiratory Disease Biomedical Research Unit at the Royal Brompton and Harefield NHS Foundation Trust and Imperial College London. The *P. aeruginosa* clinical isolate repository was established as part of the Strategic Research Centre for Pseudomonal infection in CF, funded by the Cystic Fibrosis Trust (UK). The Facility for Imaging by Light Microscopy (FILM) at Imperial College London is part-supported by funding from the Wellcome Trust (grant 104931/Z/14/Z) and BBSRC (grant BB/L015129/1).

References

1. Lee RJ, Xiong G, Kofonow JM, Chen B, Lysenko A, Jiang P, Abraham V, Doghramji L, Adappa ND, Palmer JN, Kennedy DW, Beauchamp GK, Doulias PT, Ischiropoulos H, Kreindler JL, Reed DR, Cohen NA. T2R38 taste receptor polymorphisms underlie susceptibility to upper respiratory infection. *The Journal of clinical investigation* 2012; 122: 4145-4159.
2. Kim UK, Jorgenson E, Coon H, Leppert M, Risch N, Drayna D. Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide. *Science (New York, NY)* 2003; 299: 1221-1225.
3. Meyerhof W, Batram C, Kuhn C, Brockhoff A, Chudoba E, Bufe B, Appendino G, Behrens M. The molecular receptive ranges of human TAS2R bitter taste receptors. *Chemical senses* 2010; 35: 157-170.
4. Turnbull A, Lund-Palau H, Murphy R, Simbo A, Wong K, Bush A, Alton E, J D. The T2R38 bitter taste receptor as a modifier of host response to Pseudomonas aeruginosa in cystic fibrosis: does T2R38 genotype impact on clinical infection? *Pediatric pulmonology* 2016; 51: S81-S114.
5. Turnbull A, Shoemark A, Palau HL, Murphy RA, Simbo A, Bush A, Alton EW, Davies JC. Determining the impact of the T2R38 bitter taste receptor on P. aeruginosa infection in the cystic fibrosis airway. *Journal of Cystic Fibrosis* 2017; 16: S88.
6. Shoemark A, Frost E, Dixon M, Ollosson S, Kilpin K, Patel M, Scully J, Rogers AV, Mitchison HM, Bush A, Hogg C. Accuracy of Immunofluorescence in the Diagnosis of Primary Ciliary Dyskinesia. *American journal of respiratory and critical care medicine* 2017.
7. Bolte S, Cordelieres FP. A guided tour into subcellular colocalization analysis in light microscopy. *J Microsc* 2006; 224: 213-232.
8. Lee TWR, Brownlee KG, Conway SP, Denton M, Littlewood JM. Evaluation of a new definition for chronic Pseudomonas aeruginosa infection in cystic fibrosis patients. *Journal of Cystic Fibrosis* 2003; 2: 29-34.
9. Ortori CA, Dubern JF, Chhabra SR, Camara M, Hardie K, Williams P, Barrett DA. Simultaneous quantitative profiling of N-acyl-L-homoserine lactone and 2-alkyl-4(1H)-quinolone families of quorum-sensing signaling molecules using LC-MS/MS. *Analytical and bioanalytical chemistry* 2011; 399: 839-850.
10. Shah AS, Ben-Shahar Y, Moninger TO, Kline JN, Welsh MJ. Motile cilia of human airway epithelia are chemosensory. *Science (New York, NY)* 2009; 325: 1131-1134.
11. Yan CH, Hahn S, McMahon D, Bonislawski D, Kennedy DW, Adappa ND, Palmer JN, Jiang P, Lee RJ, Cohen NA. Nitric oxide production is stimulated by bitter taste receptors ubiquitously expressed in the sinonasal cavity. *American journal of rhinology & allergy* 2017; 31: 85-92.
12. Yang J, Liu X, Yue G, Adamian M, Bulgakov O, Li T. Rootletin, a novel coiled-coil protein, is a structural component of the ciliary rootlet. *The Journal of cell biology* 2002; 159: 431-440.
13. Kerem E, Viviani L, Zolin A, MacNeill S, Hatziagorou E, Ellemunter H, Drevinek P, Gulmans V, Krivec U, Olesen H. Factors associated with FEV1 decline in cystic

fibrosis: analysis of the ECFS patient registry. *The European respiratory journal* 2014; 43: 125-133.

FIGURE LEGEND

Figure. Confocal microscopy images of nasal epithelial cells from a subject with CF. Cells were stained with antibodies to T2R38 (red), and acetylated α-tubulin (A) or rootletin (B) (both stained green). Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) (blue). Colocalized antibodies appear yellow in the merged images. Epithelial cell morphology is shown by differential interference contrast (DIC) images. T2R38 stains proximally to acetylated α-tubulin (ciliary microtubules) and colocalizes with rootletin (ciliary rootlets).

Antibodies used in these immunocytochemistry assays were: T2R38 (AB130503, Abcam, UK), acetylated α-tubulin (T6793, Sigma, UK), and rootletin (SC-374056, Santa Cruz Biotechnology, USA).

Table. *P. aeruginosa* infection category by *TAS2R38* genotype

	AVI/AVI (n=74)	AVI/PAV (n=110)	PAV/PAV n=41
Never, n(%)	4 (5)	4 (4)	3 (7)
Free, n(%)	21 (28)	36 (32)	16 (39)
Intermittent, n(%)	11 (15)	26 (24)	8 (20)
Chronic, n(%)	38 (51)	44 (40)	14 (34)

Definition of abbreviations: AVI = alanine-valine-isoleucine; PAV = proline-alanine-valine.

Impact of T2R38 receptor polymorphisms on *Pseudomonas aeruginosa* infection in cystic fibrosis

Figure

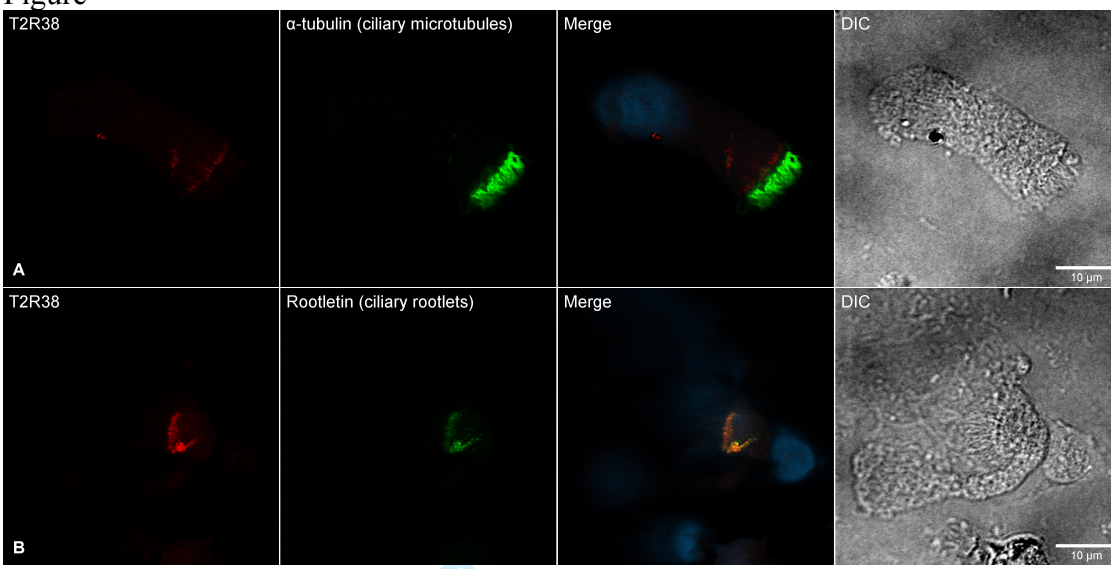

Table 2. Odds ratios for 'intermittent or chronic' *P. aeruginosa* infection by logistic regression

	Univariate		
	Odds ratio	Lower 95% CI	Upper 95% CI
Age	1.051	1.029	1.073
Sex Male* Female	 1 1.352	 0.785	 2.330
CFTR F508/F508* F508/other Other/other	 1 1.285 1.465	 0.723 0.521	 2.281 4.121
T2R38 PAV/PAV* PAV/AVI AVI/AVI	 1 1.511 1.693	 0.731 0.776	 3.125 3.694

- Baseline group for comparison of odds ratios by logistic regression.